



# Brain gamma-aminobutyric acid, but not glutamine and glutamate levels are lower in older adults with chronic musculoskeletal pain: considerations by sex and brain location

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## Abstract

**Introduction and Objectives:** GABAergic and glutamatergic neurotransmitter systems are central to the pathophysiology of chronic pain and are equally affected by aging processes. We measured levels of frontal gamma-aminobutyric acid (GABA) and the combined resonance of glutamate and glutamine (Glx) in vivo using proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) to elucidate age-specific and pain-specific associations with clinical and experimental pain in older adults.

**Methods:** Younger (18–24, n = 24) and older (60–94, n = 41) individuals part of a larger study (Neuromodulatory Examination of Pain and Mobility Across the Lifespan [NEPAL]) underwent questionnaires, quantitative sensory testing, and <sup>1</sup>H-MRS Mescher-Garwood point-resolved spectroscopy to measure GABA and Glx levels in prefrontal and sensorimotor brain regions.

**Results:** Older participants had significantly lower sensorimotor, but not prefrontal, GABA and Glx levels, compared with younger controls ( $P$ 's < 0.05). Younger controls had significantly higher prefrontal and sensorimotor GABA, but not Glx, levels compared with older controls and older adults with chronic pain ( $P$ 's < 0.05). Older males with chronic pain had significantly lower prefrontal GABA compared with older and younger male controls ( $P$ 's < 0.05). Prefrontal GABA, but not Glx, was significantly associated with self-reported and experimental pain measures ( $P$ 's < 0.05). Our results are the first to focus exclusively on age and pain differences in GABA and Glx including younger and older controls to elucidate aging and pain contributions to brain GABAergic and glutamatergic processes.

**Conclusion:** Evaluation of both the neuroinhibitory and neuroexcitatory mechanisms provide promising potential for improving both our understanding of the mechanisms of chronic pain in aging and opportunities for effective, individualized treatments.

**Keywords:** GABA, brain, MRS, musculoskeletal pain, aging

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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## 1. Introduction

Chronic pain in older individuals is a growing public health problem that negatively affects physical and cognitive function, ultimately decreasing quality of life and overall well-being.<sup>38</sup> Furthermore, effective pain treatments are lacking for this vulnerable population and the identification of potential neurobiological mechanisms underlying these pain conditions may potentially reveal effective therapeutic targets. Of particular relevance to the field of pain and aging neurobiology is the ability to measure the brain's major inhibitory and excitatory neurochemicals: gamma-aminobutyric acid (GABA) and the combined resonance of glutamate and glutamine (Glx)<sup>51</sup> using the Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) sequence<sup>32,33</sup> in proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS).<sup>11,32,35</sup> To date, this method has been used in the study of a wide range of domains, including nonnociceptive sensory processing,<sup>40</sup> depression,<sup>16</sup> Alzheimer disease,<sup>26</sup> aging,<sup>14,39</sup> and epilepsy.<sup>41</sup>

Chronic pain states also have altered brain neurochemical levels using  $^1\text{H-MRS}$ . Previous studies have shown negative associations between GABA and chronic pain.<sup>1,13,19,22,23,30,49</sup> Similarly, acute pain stimulation leads to decreases in cerebral GABA and increases in Glx levels,<sup>6</sup> although this may depend on the experimental pain modality used.<sup>28</sup> However, most of the studies to date have included both younger and older individuals, raising the possibility that in older individuals, brain aging itself, and not chronic pain, may result in altered brain biochemistry. Previous studies indicate an age-related decline in cortical GABA levels,<sup>14,17</sup> with lower frontal GABA levels being associated with worse cognitive performance.<sup>39</sup> To the best of our knowledge, this is the first report of the relationship between GABA and Glx in prefrontal (ie, a region affected by aging processes and involved in the cognitive modulation of the pain experience) and sensorimotor (ie, a brain region involved in somatosensory and pain processing) brain regions. Based on the previous literature, we hypothesized that community-dwelling older adults with chronic musculoskeletal pain would exhibit lower GABA and higher Glx levels compared with younger and older controls. In addition, we hypothesized that GABA and Glx levels would be significantly associated with self-reported and experimental pain.

## 2. Methods

### 2.1. Participants

Community-dwelling younger (18–24 years old) and older (60–93 years old) individuals who spoke and understood written English were recruited and screened for a larger cross-sectional NIH-funded study at the University of Florida focused on the neurobiology of age-related differences in pain modulation (Neuro-modulatory Examination of Pain and Mobility Across the Lifespan). Potential participants were first screened over the phone and then again in person from the fall of 2015 to fall of 2019. We excluded individuals reporting any of the following conditions either over the phone screening or during the in-person baseline session: (1) Alzheimer, Parkinson, or other diagnosis of a nervous system disorder; (2) serious psychiatric conditions (eg, schizophrenia, major depression, or bipolar disorder); (3) uncontrolled hypertension (blood pressure >150/95 mm Hg), heart failure, or history of acute myocardial infarction; (4) systemic rheumatic disorders (ie, rheumatoid arthritis, systemic lupus erythematosus, or fibromyalgia); (5) chronic opioid use; (6) magnetic resonance imaging (MRI) contraindications; (7) excessive anxiety regarding protocol procedures; (8) hospitalization within the preceding year for psychiatric illness; (9) HIV or AIDS; and (10) cognitive impairment (Modified Mini-Mental State Examination (3MS) score  $\leq 77$ ).<sup>48</sup> Younger adults were further excluded if they reported any chronic pain condition currently or in the past. All procedures were reviewed and approved by the University of Florida's Institutional Review Board, and all participants provided verbal and written informed consent. We have previously reported on various aspects of the NEPAL study participants and its detailed methodology.<sup>5,8,9,31</sup> To address the aims of this study, we included assessments of self-reported pain, depressive symptoms, a quantitative sensory testing battery, and MRS neuroimaging obtained from 3 separate laboratory visits.

#### 2.1.1. Self-reported pain

Older participants were categorized to the pain group if they reported pain on most days during the past 3 months that interfered with their life consistent with the Task Force for the Classification of Chronic Pain consensus for the 11th version of the *International Classification*

of Diseases of the World Health Organization.<sup>50</sup> Pain categorization occurred after study completion and confirmed by a medical and pain history review. Participants were also asked about their average pain intensity in the past 3 months using an 11-point numerical rating scale (0 = no pain to 10 = the most intense pain imaginable), frequency of pain during the past week, pain duration, number of anatomical pain sites, as well as pharmacological and non-pharmacological treatments used in the past 3 months as part of a standardized pain history interview.

#### 2.1.2. Depressive symptoms

The Center for Epidemiologic Studies Depression Scale questionnaire was used to measure the frequency of depressive symptoms during the past week on a 4-point Likert scale.<sup>42</sup>

#### 2.1.3. Quantitative sensory testing

Quantitative sensory testing (QST) was used to assess experimental pain sensitivity, similar to the methodology previously reported by our group in older individuals.<sup>9</sup> Standardized testing was performed at the left thenar eminence and the first metatarsal head on all participants. Thermal pain thresholds were obtained with the TSA-II NeuroSensory Analyzer and accompanying software (Medoc Ltd, Ramat Yishai, Israel) using the method of limits.

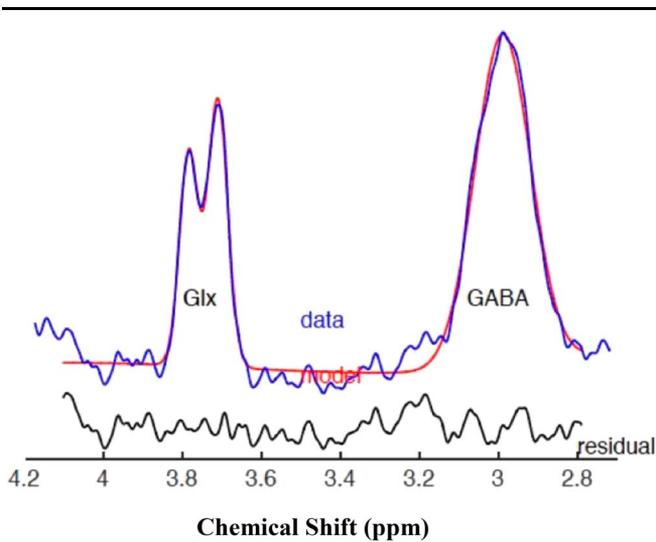
#### A Prefrontal voxel



#### B Sensorimotor voxel



**Figure 1.** Representative voxel placement from a single subject is superimposed in white, in both prefrontal (a) and sensorimotor (b) regions.



**Figure 2.** Representative spectrum for data quality purposes obtained from the Gannet software. The edited spectrum is shown in blue and overlaid in red is the model of best fit using a simple Gaussian model. Below the plot, the residual between these two is shown in black.

### 2.1.3.1. Thermal pain thresholds

Subjects were instructed to indicate as soon as the sensation changed from “just being cold to being painfully cold” or from “just being hot to being painfully hot.” Each trial began at 32°C and was either decreased (for cold pain = cold pain threshold) or increased (for heat pain = heat pain threshold) at a rate of 1.5°C/s until pain threshold was reached or the cutoff value was reached (0°C for cold pain and 50°C for heat pain). Each trial was separated by at least 20 seconds. The mean across 3 trials at each test site was calculated as the cold and heat pain thresholds.

### 2.1.3.2. Pressure pain thresholds

A handheld digital pressure algometer (AlgoMed; Medoc) was applied to the quadriceps and trapezius muscles at a constant rate of 30 kPa/s up to a maximum pressure level of 1000 kPa. Participants indicated when the pressure first became painful by pressing a button. The order of testing site was counterbalanced. Each trial was repeated 3 times at each site and averaged.

## 2.2. Z-transformation of quantitative sensory testing data

Older adult’s QST data were standardized by using the following expression<sup>18</sup>:  $Z\text{-score} = (X_{\text{single older subject}} - \text{Mean}_{\text{younger controls}}) / \text{SD}_{\text{younger controls}}$ .

This procedure results in a QST profile with zero mean, 1SD. For clarity of data presentation, we adjusted the algebraic sign of Z-score values so that it reflects the participant’s sensitivity. Z-values above “0” indicates higher pain sensitivity, whereas Z-scores below “0” indicate lower pain sensitivity.

### 2.2.1. Neuroimaging session

Magnetic resonance imaging data were collected at the McKnight Brain Institute on the Advanced Magnetic Resonance Imaging and Spectroscopy facility’s Philips 3-Tesla scanner using a 32-channel radiofrequency coil. Participants were asked about their current pain intensity on the MRI table before starting the scanning procedures using a visual analogue scale (0–100). A T1-weighted anatomical image (magnetization-prepared rapid gradient-echo; repetition time/echo time = 7.2 ms/3.7 ms, 1-mm<sup>3</sup> isotropic voxels) was acquired for MRS voxel placement and segmentation. GABA-edited MRS data were acquired using the MEGA-PRESS sequence.<sup>32</sup> The prefrontal voxel was placed medial on the axial plane, aligned with the genu of the corpus callosum, inclusive of the pregenual ACC and medial prefrontal cortex (ie, mainly Brodmann areas 10 [ie, the anterior-most portion of the prefrontal cortex] and 32). When time allowed, we also collected data from the sensorimotor (S/M1) regions with the voxel centered over the hand knob area,<sup>55</sup> parallel to the anterior–posterior axis. All voxels were 3 × 3 × 3 cm<sup>3</sup> and representative voxel locations are shown in **Figure 1**. As part of the quality control procedures, voxel locations were verified after data collection to identify potential placement errors. Editing was performed with 14-ms sinc-Gaussian pulses applied at 1.9 ppm in the on experiment and 7.46 ppm in the off experiment. This editing scheme co-edits approximately 50% macromolecules at 3 ppm, which are coupled to spins at 1.7 ppm also inverted by editing pulses. Therefore, all GABA values reported refer to GABA+ macromolecules. Acquisition details were repetition time per echo time of 2 seconds/68 ms, 320 transients with on-off scans alternating every 2 transients, a 16-step phase cycle (with steps repeated for on and off), 2048 data points acquired at a spectral width of 2 kHz, and variable pulse power and optimized relaxation delays water suppression.<sup>20</sup> Quantitative analysis was performed using the Gannet program (version 2.0).<sup>12</sup> All time domain data were frequency-corrected and phase-corrected using spectral registration,<sup>36</sup> filtered with a 3-Hz exponential line broadening and zero filled by a factor of 16. Metabolite levels were fitted in the difference spectrum using simultaneous GABA + glutamate and glutamine (Glx) signal fitting and both were acceptable<sup>34</sup> (**Fig. 2**). The GABA peak was fit using a 3-Gaussian function and a nonlinear baseline with the Glx signal at 3.75 ppm fit using nonlinear least squares fitting. The metabolite peaks were quantified relative to water (fit with a

**Table 1**

**Tissue composition in the voxels across participants.**

	Younger controls	Older controls	Older chronic pain	<i>P</i>
Prefrontal voxel	n = 24	n = 12	n = 29	
Gray matter fraction	0.586 ± 0.03	0.489 ± 0.04	0.505 ± 0.04	<b>0.000</b>
White matter fraction	0.288 ± 0.04	0.280 ± 0.04	0.287 ± 0.05	0.851
Cerebrospinal fluid fraction	0.125 ± 0.03	0.231 ± 0.07	0.208 ± 0.05	<b>0.000</b>
Sensorimotor voxel	n = 12	n = 5	n = 14	
Gray matter fraction	0.293 ± 0.05	0.216 ± 0.02	0.263 ± 0.03	<b>0.003</b>
White matter fraction	0.640 ± 0.06	0.644 ± 0.06	0.604 ± 0.05	0.234
Cerebrospinal fluid fraction	0.067 ± 0.02	0.140 ± 0.04	0.133 ± 0.05	<b>0.000</b>

Bold entries represent probability values less than 0.05.

**Table 2**  
Paired correlations between metabolite levels at the prefrontal and the sensorimotor voxels in a subset of participants (n = 31).

	Paired correlation	Probability
CSF-corrected GABA	0.026	0.894
Tissue-corrected GABA	−0.058	0.769
CSF-corrected Glx	0.380	0.046
Tissue-corrected Glx	0.368	0.054

GABA, gamma-aminobutyric acid; Glx, glutamate and glutamine.

Gaussian–Lorentzian model) in institutional units. The large interindividual variability in healthy brain aging processes (Table 1), even across regions (Table 2), necessitates the consideration of voxel tissue composition to ensure that metabolite changes are not unduly affected by the composition of the measurement voxel.<sup>21</sup> In this study, 2 distinct approaches were implemented: (1) the most commonly used approach, the cerebrospinal fluid (CSF) correction that normalizes the voxel by the non-CSF voxel fraction (1-fCSF), with the implicit assumption that the metabolites are not contained in CSF or, if present, do not contribute to the MRS signal, and (2) a more recently proposed tissue correction, the  $\alpha$  correction that accounts for the different concentrations of GABA and Glx in the gray and white matter in addition to accounting for differential tissue relaxations and signal constants to better address the underlying dependency of metabolites on the tissue composition of the voxel.<sup>21</sup> These corrections involved the generation of a binary mask of the MRS voxel created with the same imaging matrix as the T1-weighted anatomical image, using Gannet tool GannetCoRegister,<sup>21</sup> integrated voxel-to-image co-registration. Segmentation of the anatomical image was performed using GannetSegment<sup>21</sup> and

Segment in SPM12,<sup>2</sup> which was used to obtain average coordinates of the center of ROIs in [X,Y,Z] format (prefrontal: [0.98, 47.96, 1.89] and S1/M1: [31.06, −19.53, 46.1]).

### 2.3. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 27. Descriptive statistics were examined across groups using *t*-tests for continuous variables and the  $\chi^2$  test for nominal variables. Factorial analyses of covariance were used to determine differences in metabolite levels between groups with sex as a secondary between-subject factor while accounting for race. Partial correlation analyses controlling for age, sex, and race were used to assess associations between brain metabolite levels with clinical and experimental variables. A probability less than 0.05 was set a priori as statistically significant with a false discovery rate correction using Benjamini–Hochberg probabilities. We also evaluated significance using 95% bootstrapped confidence intervals based on 5000 bootstrap samples in all procedures. Partial eta squared was also reported to assess the magnitude of the differences where small, medium, and large effect sizes are represented by 0.01, 0.06, and 0.14, respectively.<sup>7</sup>

### 3. Results

A total of 186 younger and older individuals were screened with 84 participants being eligible for neuroimaging and undergoing the MRS MEGA-PRESS sequence acquisition. A number of MEGA-PRESS sequences failed preprocessing quality control measures in the prefrontal (n = 17) and sensorimotor (n = 23) regions. Of these 67 participants, 2 older adults (1 reporting chronic pain and 1 not reporting pain) were excluded as their

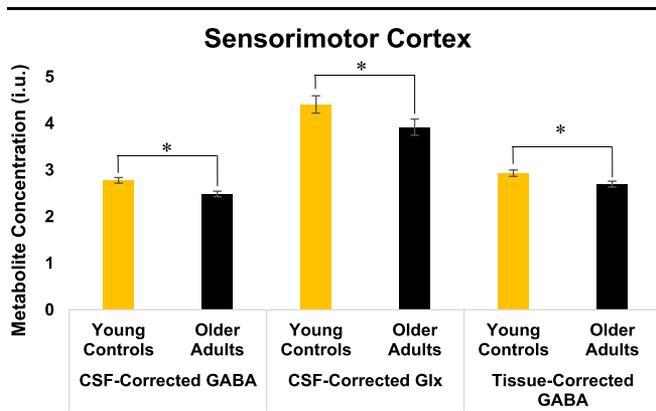
**Table 3**  
Differences in demographic and clinical characteristics between the groups.

	Younger controls (n = 23)	Older controls (n = 12)	Older chronic pain (n = 29)	P
Age, mean $\pm$ SD	21.7 $\pm$ 1.8	73.7 $\pm$ 6.7	72.0 $\pm$ 7.1	<b>0.001*</b>
Sex, no.				0.458†
Male	11	5	9	
Female	12	7	20	
Race, no.				<b>0.020†</b>
Caucasian	13	12	26	
African American	1	0	2	
Asian	1	0	0	
Pacific Islander	1	0	1	
Hispanic	7	0	0	
Education, mean $\pm$ SD	14.9 $\pm$ 1.5	16.4 $\pm$ 2.9	15.4 $\pm$ 2.6	0.187*
CES-D, mean $\pm$ SD	7.6 $\pm$ 6.1	7.4 $\pm$ 3.3	7.9 $\pm$ 5.6	0.969*
No. of anatomical pain sites, mean $\pm$ SD	—	—	4.1 $\pm$ 2.3	—
Current pain at MRI (0–100), mean $\pm$ SD	0.9 $\pm$ 4.1	0.9 $\pm$ 2.3	13.6 $\pm$ 16.0	<b>0.001*</b>
Ave. pain in the past 3 months (0–10), mean $\pm$ SD	0 $\pm$ 0	0 $\pm$ 0	4.8 $\pm$ 2.1	<b>0.001*</b>
Pain days in past week (0–7), mean $\pm$ SD	—	—	5.4 $\pm$ 2.2	—
Pain duration, mean $\pm$ SD	—	—	10.5 $\pm$ 2.1	—
Narcotic (PRN), no. (%)	—	—	5 (17.2)	—
NSAID/Acetaminophen, no. (%)	—	—	16 (55.2)	—
Nonpharmacological interventions, no. (%)	—	—	18 (62.1)	—

\* Analysis of variance tests. Bold entries represent probability values less than 0.05.

†  $\chi^2$  tests.

CES-D, Center for Epidemiologic Studies Depression Scale; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drugs; PRN, as needed.



**Figure 3.** Differences in sensorimotor GABA and Glx metabolite levels between older and younger participants. \*Statistically significant post hoc comparisons at a probability less than 0.05. GABA, gamma-aminobutyric acid

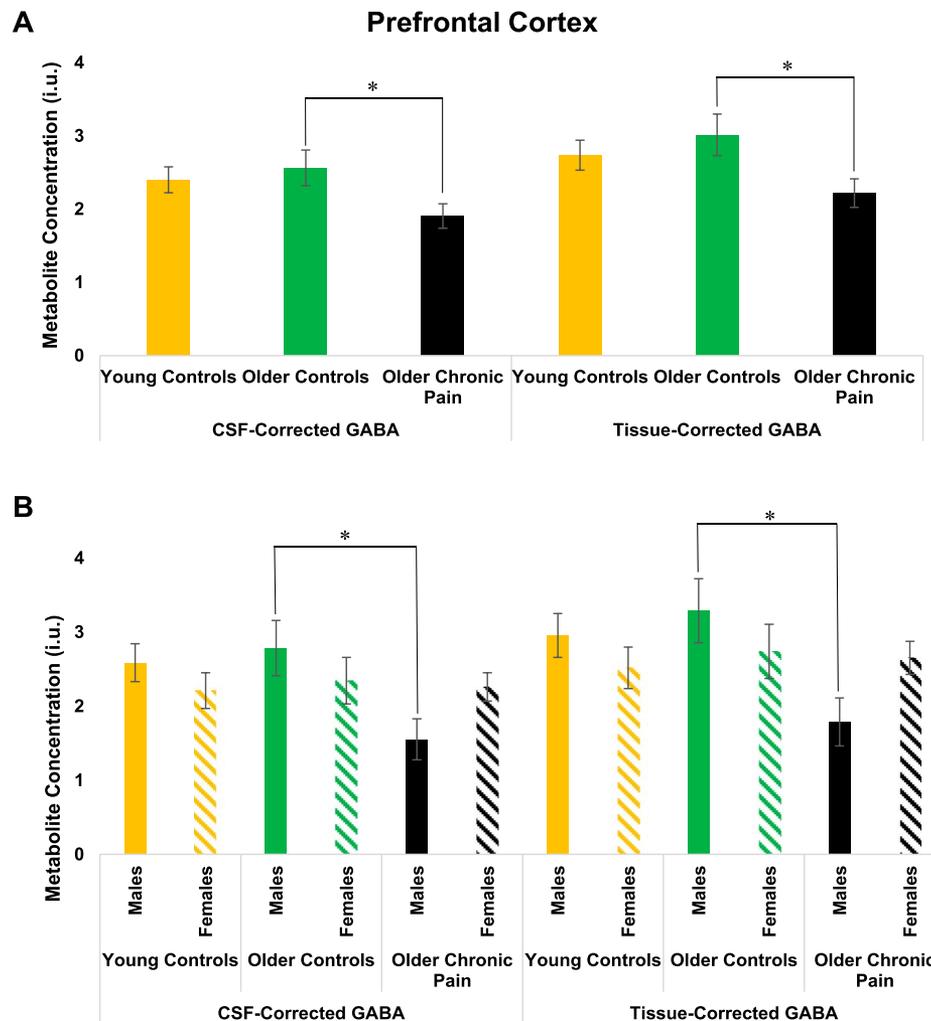
GABA levels were significantly greater than 3 SDs from the sample mean, whereas 1 younger adult reported a chronic pain condition (ie, migraine headaches). Thus, this study includes 64 participants with high-quality MRS data in a prefrontal voxel. In a subset ( $n = 31$ ), data from a second voxel in the sensorimotor

cortex were also obtained. Participants were between the ages of 19 and 94 years and were mostly non-Hispanic White (80.3%) and female (60.7%). Most of our older adults (65.9%) reported chronic musculoskeletal pain during the past 3 months with 2 pain problems on average that were most commonly reported in the legs or knees (85.2%) and back (55.6%) regions. Approximately 31% of participants ( $n = 9$ ) reported their pain to be constant or continuous while 48% of participants ( $n = 14$ ) reported their pain to be intermittent and 20% reported their pain had no predictable pattern ( $n = 6$ ). Summary demographic and clinical characteristics are presented in **Table 3**.

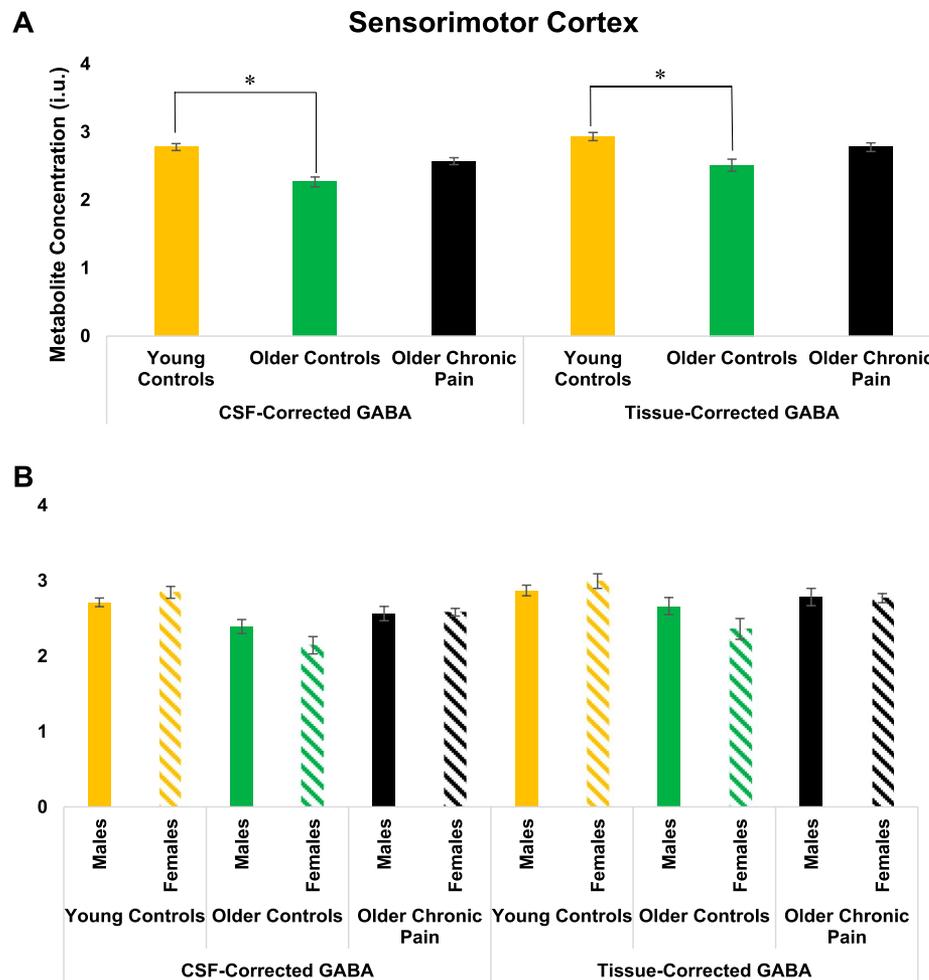
### 3.1. Age differences in gamma-aminobutyric acid and glutamate and glutamine metabolite levels

#### 3.1.1. Prefrontal CSF-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 64$ )

There was a nonsignificant main effect of Age ( $F(1,59) = 1.3$ ,  $P = 0.253$ , effect size = 0.022) and Age by Sex interaction ( $F(1,59) = 2.1$ ,  $P = 0.155$ , effect size = 0.034) in prefrontal GABA. Similarly, there was a nonsignificant main effect of Age ( $F(1,59) = 1.1$ ,  $P = 0.304$ , effect size = 0.109) and Age by Sex interaction ( $F(1,59) = 0.3$ ,  $P = 0.609$ , effect size = 0.005) in prefrontal Glx.



**Figure 4.** Adjusted differences in CSF-corrected and tissue-corrected prefrontal GABA metabolite levels between the groups and stratified by sex. \*Statistically significant post hoc comparisons at a probability less than or equal to 0.05. GABA, gamma-aminobutyric acid



**Figure 5.** Adjusted differences in CSF-corrected and tissue-corrected sensorimotor GABA metabolite levels between the groups. \* Statistically significant post hoc comparisons at a probability less than 0.05. There was no statistically significant Pain by Sex interactions ( $p$ 's > 0.05). GABA, gamma-aminobutyric acid

### 3.1.2. Prefrontal tissue-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 64$ )

There was a nonsignificant main effect of Age ( $F(1,59) = 0.8$ ,  $P = 0.369$ , effect size = 0.014) and Age by Sex interaction ( $F(1,59) = 2.1$ ,  $P = 0.150$ , effect size = 0.035) in prefrontal GABA. Similarly, there was a nonsignificant main effect of Age ( $F(1,59) = 0.6$ ,  $P = 0.431$ , effect size = 0.011) and Age by Sex interaction ( $F(1,59) = 0.3$ ,  $P = 0.599$ , effect size = 0.005) in prefrontal Glx.

### 3.1.3. Sensorimotor CSF-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 31$ )

There was a significant main effect of Age ( $F(1,26) = 13.4$ ,  $P = 0.001$ , effect size = 0.340) where older adults ( $n = 19$ ) had lower sensorimotor GABA ( $M = 2.49$ ,  $SEM = 0.05$ ) compared with younger controls ( $n = 12$ ,  $M = 2.78$ ,  $SEM = 0.06$ , bootstrapped  $P = 0.002$ ). There was no Age by Sex interaction in sensorimotor GABA ( $F(1,26) = 0.4$ ,  $P = 0.540$ , effect size = 0.015). There was a significant main effect of Age group ( $F(1,26) = 5.4$ ,  $P = 0.028$ , effect size = 0.172) where older adults ( $n = 19$ ) had lower sensorimotor Glx ( $M = 3.9$ ,  $SEM = 0.1$ ) compared with younger controls ( $n = 12$ ,  $M = 4.4$ ,  $SEM = 0.2$ , bootstrapped  $P = 0.024$ , **Fig. 3**). There was no Age by Sex interaction in sensorimotor Glx ( $F(1,26) = 0.3$ ,  $P = 0.561$ , effect size = 0.013).

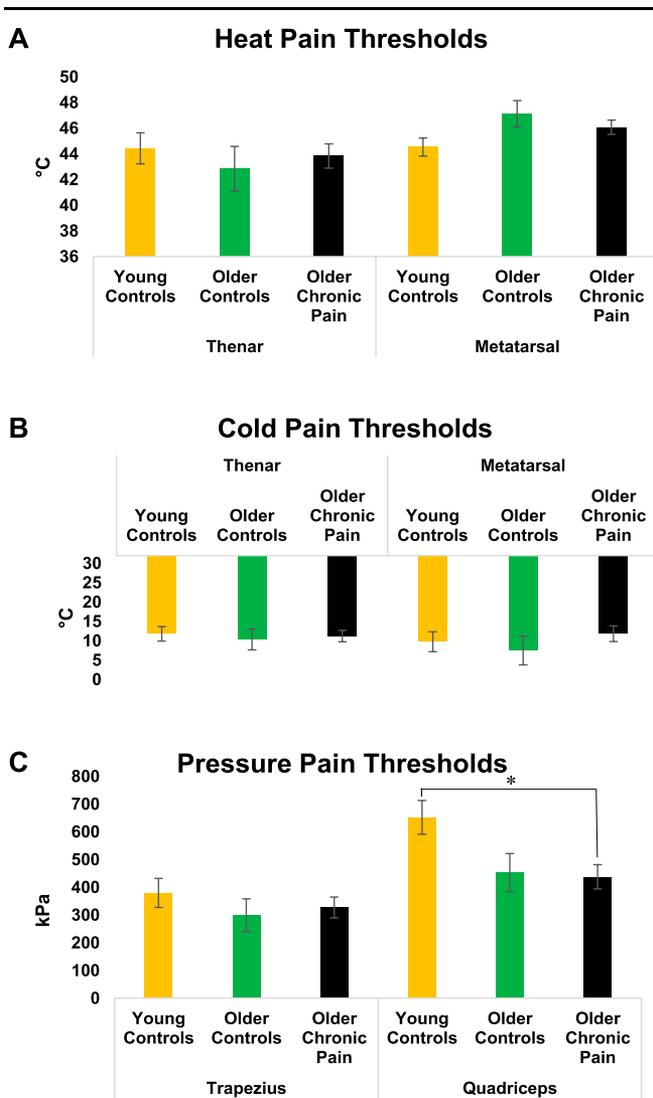
### 3.1.4. Sensorimotor tissue-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 31$ )

There was a significant main effect of Age ( $F(1,26) = 6.4$ ,  $P = 0.018$ , effect size = 0.197) where older adults ( $n = 19$ ) had lower sensorimotor GABA ( $M = 2.70$ ,  $SEM = 0.05$ ) compared with younger controls ( $n = 12$ ,  $M = 2.93$ ,  $SEM = 0.07$ , bootstrapped  $P = 0.037$ , **Fig. 3**). There was no Age by Sex interaction in sensorimotor GABA ( $F(1,26) = 0.5$ ,  $P = 0.470$ , effect size = 0.023). There was a nonsignificant main effect of Age ( $F(1,26) = 3.1$ ,  $P = 0.088$ , effect size = 0.108) and Age by Sex interaction in sensorimotor Glx ( $F(1,26) = 0.2$ ,  $P = 0.649$ , effect size = 0.008).

## 3.2. Age and pain differences in gamma-aminobutyric acid and glutamate and glutamine metabolite levels

### 3.2.1. Prefrontal CSF-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 64$ )

There was a significant main effect of Age-Pain (ie, comparing the 3 groups: younger controls, older controls, and older adults reporting pain) ( $F(2,57) = 3.2$ ,  $P = 0.045$ , effect size = 0.103). Older individuals with chronic pain had significantly lower prefrontal GABA ( $M = 1.9$ ,  $SEM = 0.2$ ) compared with older controls ( $M = 2.6$ ,  $SEM = 0.2$ , bootstrapped  $P = 0.027$ , **Fig. 4**). There was a trend for Age-Pain by Sex interaction ( $F(2,57) = 3.1$ ,  $P = 0.050$ , effect size = 0.100, **Figure 4**) in prefrontal GABA. There was a



**Figure 6.** Experimental pain thresholds by study group and test site where only pressure pain thresholds in the quadriceps survived post hoc corrections ( $P < 0.05$ ). \*Statistically significant post hoc comparison at a probability less than 0.05.

nonsignificant main effect of Age-Pain group ( $F(2,57) = 0.5$ ,  $P = 0.583$ , effect size = 0.019) or Age-Pain by Sex interaction ( $F(2,57) = 0.2$ ,  $P = 0.807$ , effect size = 0.008) in prefrontal Glx.

### 3.2.2. Prefrontal tissue-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 64$ )

There was a significant main effect of Age-Pain ( $F(2,57) = 3.2$ ,  $P = 0.049$ , effect size = 0.101). Older individuals with chronic pain had significantly lower prefrontal GABA ( $M = 2.2$ ,  $SEM = 0.2$ ) compared with older controls ( $M = 3.0$ ,  $SEM = 0.3$ , bootstrapped  $P = 0.021$ , **Fig. 4**). There was a significant Age-Pain by Sex interaction ( $F(2,57) = 3.2$ ,  $P = 0.039$ , effect size = 0.108) in prefrontal GABA where older males with chronic pain had significantly lower prefrontal GABA ( $M = 1.8$ ,  $SEM = 0.3$ ) compared with older ( $M = 3.3$ ,  $SEM = 0.4$ , bootstrapped  $P = 0.022$ ) and younger male controls ( $M = 3.0$ ,  $SEM = 0.3$ , bootstrapped  $P = 0.028$ , **Fig. 4**). There was a nonsignificant main effect of Age-Pain ( $F(2,57) = 0.3$ ,  $P = 0.711$ , effect size = 0.012) or Age-Pain by Sex interaction ( $F(2,57) = 0.3$ ,  $P = 0.768$ , effect size = 0.010) in prefrontal Glx.

### 3.2.3. Sensorimotor CSF-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 31$ )

There was a significant main effect of Age-Pain ( $F(2,24) = 16.8$ ,  $P < 0.001$ , effect size = 0.583) where younger controls had significantly higher sensorimotor GABA ( $M = 2.8$ ,  $SEM = 0.1$ ) compared to older controls ( $M = 2.3$ ,  $SEM = 0.1$ , bootstrapped  $P = 0.001$ ) and older participants with chronic pain ( $M = 2.6$ ,  $SEM = 0.1$ , bootstrapped  $P = 0.020$ , **Fig. 5**). There was not a significant Age-Pain by Sex interaction ( $F(2,24) = 2.3$ ,  $P = 0.120$ , effect size = 0.162) in sensorimotor GABA. There was a nonsignificant main effect of Age-Pain ( $F(2, 24) = 2.6$ ,  $P = 0.098$ , effect size = 0.176) or Age-Pain by Sex interaction ( $F(2,24) = 0.2$ ,  $P = 0.794$ , effect size = 0.019) in sensorimotor Glx.

### 3.2.4. Sensorimotor tissue-corrected gamma-aminobutyric acid and glutamate and glutamine ( $n = 31$ )

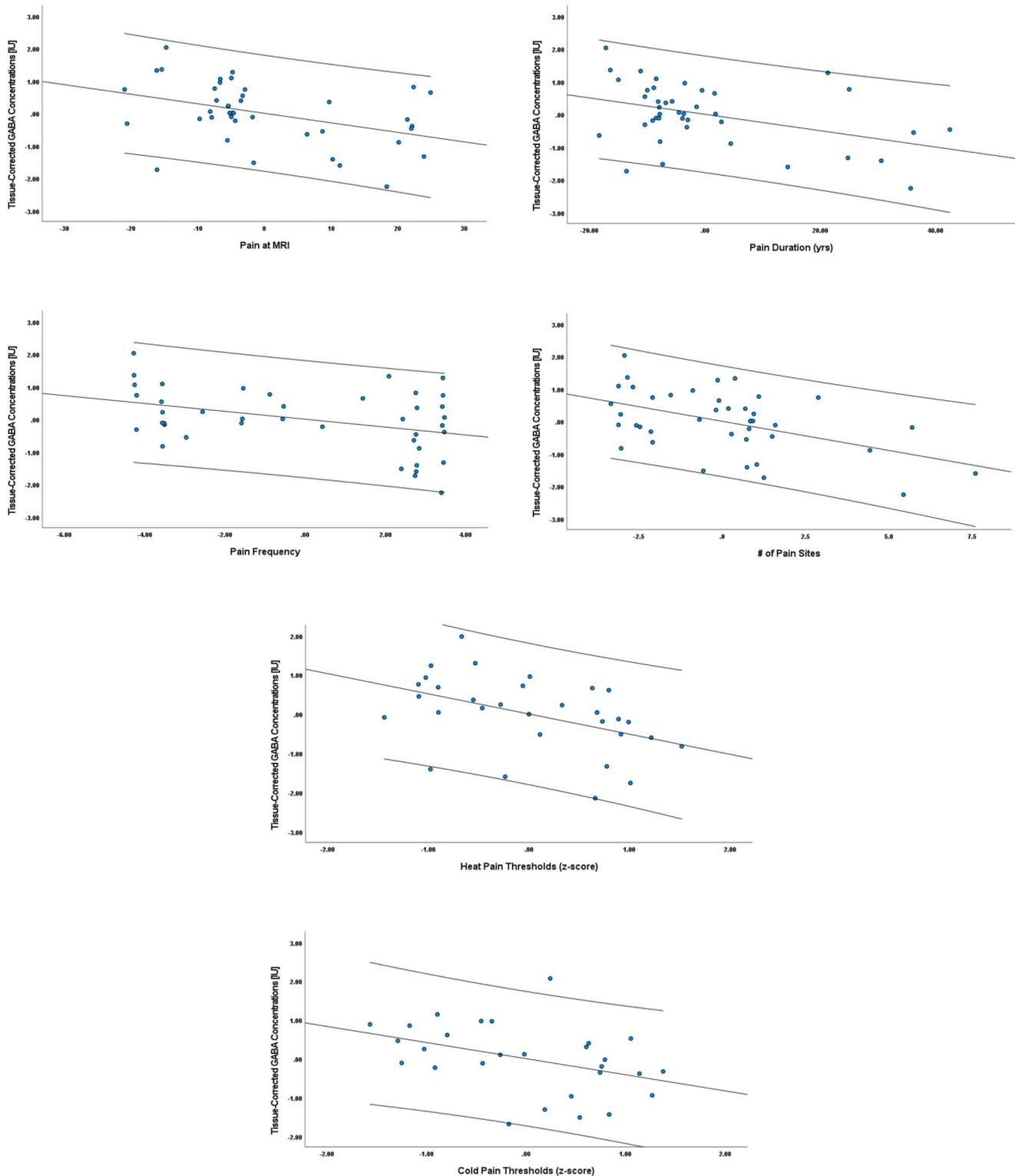
There was a significant main effect of Age-Pain ( $F(2,24) = 7.4$ ,  $P = 0.004$ , effect size = 0.414) where older controls had significantly lower sensorimotor GABA ( $M = 2.4$ ,  $SEM = 0.1$ ) compared with younger controls ( $M = 2.9$ ,  $SEM = 0.1$ , bootstrapped  $P = 0.008$ , **Fig. 5**). There was not a significant Age-Pain by Sex interaction ( $F(2,24) = 0.6$ ,  $P = 0.576$ , effect size = 0.051) in sensorimotor GABA. There was a nonsignificant main effect of Age-Pain ( $F(2,24) = 1.5$ ,  $P = 0.254$ , effect size = 0.122) or Age-Pain by Sex interaction ( $F(2,24) = 0.5$ ,  $P = 0.596$ , effect size = 0.048) in sensorimotor Glx.

### 3.3. Associations between prefrontal gamma-aminobutyric acid and glutamate and glutamine levels with clinical and experimental pain in older adults

Experimental pain thresholds by study group and test site are presented in **Figure 6**. Post hoc comparisons revealed older individuals with chronic pain had significantly lower pressure pain thresholds in the quadriceps ( $M = 426.1$ ,  $SEM = 43.9$ ) compared with young controls ( $M = 666.5$ ,  $SEM = 63.3$ , bootstrapped  $P = 0.006$ , **Fig. 6**). Prefrontal GABA metabolite levels were inversely correlated with self-reported pain variables surviving both multiple comparison corrections as well as bootstrapping procedures controlling for race, sex, and age of the participants. Similarly, heat and cold pain thresholds were inversely associated with prefrontal GABA levels and these associations were supported by bootstrapping procedures (**Fig. 7**). However, cold pain thresholds did not survive multiple comparison corrections. Prefrontal GABA levels were not associated with pressure pain thresholds. Prefrontal Glx levels were not significantly associated with any of the variables of interest. Findings are summarized in **Table 4**. Owing to the small number of participants who had data in the sensorimotor voxel and self-reported and experimental pain measures, we did not examine associations between these variables.

## 4. Discussion

This study aimed to determine self-reported and experimental pain-related associations in brain levels of GABA and Glx, reflective of inhibitory and excitatory tone in community-dwelling older adults. Several key findings emerged. First, older adults with chronic pain had significantly lower prefrontal GABA levels that were driven, in part, by older males with chronic pain having lower levels of prefrontal GABA compared with younger and older male controls. Second, prefrontal, but not sensorimotor, GABA levels were associated with greater self-reported pain, greater pain



**Figure 7.** Adjusted associations between tissue-corrected prefrontal GABA levels with clinical and experimental pain variables (QST variables as z-scores) controlling for age, sex, and race. GABA, gamma-aminobutyric acid; QST, quantitative sensory testing.

frequency and duration, greater number of pain sites, and greater thermal pain sensitivity. Finally, prefrontal Glx levels were not associated with any self-reported or experimental pain characteristics in our older sample. Our findings suggest a key imbalance among inhibitory systems that may contribute to the increased prevalence of chronic pain in older individuals.

Our investigation furthers our understanding of the GABAergic and glutamatergic systems in the context of pain and aging brain processes. To the best of our knowledge, this is the first study to simultaneously examine clinical and experimental pain and their associations with GABA and Glx levels in a community-dwelling sample. Glutamate and GABA systems are key signal transducers in

**Table 4**  
**Partial correlation coefficients assessing associations between prefrontal gamma-aminobutyric acid and glutamate and glutamine metabolite levels with clinical and experimental pain measures in older participants controlling for race, age, and sex.**

	<b>r, P</b>	<b>Benjamini-Hochberg P</b>	<b>Bootstrapped confidence intervals*</b>
Pain at MRI			
GABA	<b>r = -0.407, P = 0.011</b>	<b>0.045</b>	<b>-0.679, -0.044</b>
Glx	r = -0.041, P = 0.814	0.938	-0.315, 0.324
Pain frequency			
GABA	<b>r = -0.392, P = 0.015</b>	<b>0.045</b>	<b>-0.642, -0.078</b>
Glx	r = 0.020, P = 0.908	0.938	-0.312, 0.462
Pain duration			
GABA	<b>r = -0.415, P = 0.010</b>	<b>0.045</b>	<b>-0.722, -0.030</b>
Glx	r = 0.021, P = 0.905	0.938	-0.255, 0.528
No. of pain sites			
GABA	<b>r = -0.490, P = 0.002</b>	<b>0.028</b>	<b>-0.711, -0.170</b>
Glx	r = 0.019, P = 0.910	0.938	-0.157, 0.296
Heat pain thresholds			
GABA	<b>r = -0.451, P = 0.016</b>	<b>0.045</b>	<b>0.710, 0.137</b>
Glx	r = -0.121, P = 0.549	0.938	-0.476, 0.258
Cold pain thresholds			
GABA	<b>r = -0.408, P = 0.043</b>	0.100	<b>0.127, 0.714</b>
Glx	r = -0.017, P = 0.938	0.938	-0.332, 0.420
Pressure pain thresholds			
GABA	r = 0.088, P = 0.632	0.938	-0.382, 0.473
Glx	r = -0.093, P = 0.625	0.938	-0.431, 0.343

\* Results are based on 5000 bootstrap samples. Bold entries represent probability values less than 0.05. GABA, gamma-aminobutyric acid; Glx, glutamate and glutamine; MRI, magnetic resonance imaging.

pain-processing pathways and are involved in various pain-gating mechanisms.<sup>4,15,23,27,29,52,53</sup> Lower GABA levels in our older adults with chronic musculoskeletal pain are consistent with the existing literature using <sup>1</sup>H-MRS where reduced cerebral GABA levels have been reported in fibromyalgia<sup>13</sup> and chronic pelvic pain.<sup>19</sup> Specifically, it seems that our differences were driven, in part, by the older male participants with chronic pain. To the best of our knowledge, such pain by sex differences have not been previously reported in humans, and multiple mechanisms may account for these findings. For example, lower GABA<sub>A</sub>R α3 subunit expression has been reported in older vs younger males that could account for GABAergic system dysfunction.<sup>37</sup> Indeed, work in mice expressing genetically engineered GABA<sub>A</sub> receptor α subunits has shown that α2 and α3 GABA<sub>A</sub> receptors are the most relevant GABA<sub>A</sub> receptor subtypes for spinal analgesia.<sup>27,44</sup> Future studies are needed to replicate the sex by pain interactions in the GABAergic system in older adults.

Moreover, lower GABA levels were associated with greater self-reported pain, greater pain frequency and duration, greater number of reported pain sites, and greater thermal pain sensitivity. These findings are consistent with previous research where GABA levels correlate with clinical pain severity<sup>45</sup> and experimental pain sensitivity.<sup>49</sup> In healthy younger individuals, acute experimental pain stimulation has been found to change GABA levels in various brain regions.<sup>6,28</sup> Specifically, administration of painful stimulation momentarily decreased GABA levels in the anterior cingulate and occipital cortex,<sup>6</sup> and tonic heat pain stimulation increased GABA levels in the prefrontal cortex. Although the above studies link cerebral GABA levels with chronic and acute pain processing, the directionality of the findings is less consistent. However, our findings are in sharp contrast to findings in persons with migraine<sup>1</sup> and previous studies reporting associations between Glx concentrations and pain characteristics.<sup>46,52</sup> This is likely due to the different brain regions investigated, different MRS acquisition and analyses parameters, and the age of the included participants. Most of the studies mentioned above have only included healthy younger

individuals or a mixture of younger and older adults (except<sup>45</sup> in knee osteoarthritis), which could account for these discrepancies because there are significant age-related changes in brain metabolite levels.<sup>3,14,17,39</sup> In addition, we used a large prefrontal ROI covering several anatomically distinct brain areas, which are involved not only in descending inhibition but also in cognitive modulation through inhibitory GABAergic neurons. Furthermore, previous basic research showed hyperactivity in the amygdala leads to deactivation of prefrontal regions<sup>24</sup> through GABAergic mechanisms emphasizing the need for studies in animal models.<sup>25</sup> Future larger studies in humans must also differentiate between pain conditions in older vs younger populations and include appropriate age-control groups to examine metabolite levels across various regions.<sup>10</sup>

Our study is limited by its small sample size, particularly with respect to older controls. This is not surprising given the high prevalence of chronic pain in older individuals, which further highlights the need to *identify* and study older adults without any chronic pain. Thus, future investigations in older adults should identify these true "control" groups. Second, the source of the Glx and GABA levels measured cannot be ascertained using <sup>1</sup>H-MRS. Glx levels reflect the combination of glutamate (Glu) and glutamine (Gln) levels (ie, Glx = Glu + Gln). Thus, we cannot determine whether the Gln and Glu levels are in opposing directions or evaluate their levels separately. On the other hand, the GABA measured using <sup>1</sup>H-MRS is believed to represent mostly GABA linked to the "tonic" or GABAergic inhibitory tone of a localized brain region.<sup>43,47</sup> Nonetheless, the <sup>1</sup>H-MRS signal arises from the gray and white matter and is an ensemble average of multiple different cell types, including astrocytes, glia, and neurons and has been reported across a number of domains to be associated with increased inhibitory capacity.<sup>54</sup> Therefore, the metabolite signal might be at regions distant from the synapse. Animal studies are needed to determine the exact source and mechanism underlying the association of pain with these metabolites. Third, this is a cross-sectional study and no causal inferences can be made. It is not known whether younger and older individuals have predisposing

metabolite differences or whether the experience of pain or aging leads to such metabolite imbalances. Fourth, our older sample may not be representative of the general aging population because it included mostly Caucasian community-dwelling older individuals, who were cognitively intact and generally healthy. Longitudinal and basic studies including larger, more diverse samples with more severe pain and disability are needed to answer these questions in the context of an aging nervous system. Fifth, it is possible that our findings are driven by differences in momentary pain ratings, and future studies that test individuals with chronic pain when pain-free are needed to determine these associations. Furthermore, future studies should be designed to test whether medications taken by the study participants could drive potential metabolite differences, although our study participants were not taking medications affecting the GABAergic systems. Finally, given the small sample collected in the sensorimotor cortex, these findings, including the null findings, should be considered preliminary and require replication.

Despite these limitations, our study is the first to focus exclusively on age and pain differences including younger and older control cohorts, which is important to try to elucidate independent aging and pain contributions to brain GABAergic and glutamatergic processes. The present work includes 2 key neurochemicals to the forefront of our thinking regarding the molecular processes involved in chronic pain in older individuals. Our study supports prefrontal cortex GABA as a potential marker of pain severity and processing in chronic musculoskeletal pain states independent of age-related decline in these neurochemical systems. These findings may support the idea that GABAergic disinhibition in pain-processing brain networks may contribute to pain development and chronification in older individuals.<sup>29</sup> Finally, these data may also indicate that pharmacologic interventions that specifically target GABAergic neurotransmission may be an effective target in these older individuals with chronic musculoskeletal pain and lower brain GABA levels.

## Disclosures

The authors have no conflicts of interest to declare.

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